RESEARCH ARTICLE

Edible nanogel carrageenan/mushrooms stipe prolong mushrooms harvest period

Han Wang[1, 2], Jingru Wang[3], Rong Wang[3], Hongge Shi[4], Li Hu[2], Jiangmian Wei[2], Yufang Li[2], Hongzhou Zhang[5], Junfeng Qi[1, 2, 5, *], Wansen Li[1, *]

[1]Affiliated Central Hospital of Huanghuai University, Zhumadian, Henan, China. [2]School of Biological and Food Engineering, Huanghuai University, Zhumadian, Henan, China. [3]Zhumadian Tobacco Company of Henan Province, Zhumadian, Henan, China. [4]Zhumadian Academy of Agricultural Sciences, Zhumadian, Henan, China. [5]China Meheco Topfond Pharmaceutical Co., Ltd., Zhumadian, Henan, China.

Received: December 18, 2024; **accepted:** May 5, 2025.

Edible fungi have become increasingly important as an alternative food source worldwide with consumption showing sustained growth. Shiitake mushrooms occupy a prominent market position due to its unique sensory properties, rich nutritional value, and potential health benefits. However, fresh *Lentinus edodes* are susceptible to postharvest water loss and microbial decomposition, leading to a decline in quality and a reduction in shelf life, which poses a major constraint for industrial-scale production. To address this challenge, this research focused on extending the shelf life of *Lentinus edodes* by using a carrageenan/*Lentinus edodes* stipe-based edible nanogel to ensure its nutritional value and food safety. The results showed that as the stipe content in the nanofiber coating increased, the coating slowed the respiration rate and inhibited the water loss, while the antioxidant activity increased. During storage, the hardness, total soluble solids, total phenolic content, antioxidant activity, and total flavonoid content of *Lentinus edodes* were better maintained than those in uncoated control samples. On day 12, with increasing mushroom stipe content in the coating, the contents of total phenols, flavonoids, and superoxide dismutase increased from 1.59 × 10^{-1} to 2.30 × 10^{-1} mg/g, 0.59 × 10^{-1} to 0.74 × 10^{-1} g/kg, and 3.30 × 10^{-1} to 4.83 × 10^{-1}, respectively, while 1,1-Diphenyl-2-picrylhydrazyl radical 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl (DPPH) scavenging activity increased from 24.54 to 49.32%. The results suggested that double-layer edible nanogel, i.e. carrageenan gel mixed with mushroom stipe, could be an alternative and less expensive method to extend the shelf life of mushrooms up to 12 days when compared to uncoated samples. This research has encouraged scientists to further explore new preservation technologies and packaging materials, which not only extend the shelf life of food, but also enhance its quality and nutritional value.

Keywords: mushrooms stipe; carrageenan; edible nanogel; prolong harvest period.

[*]**Corresponding authors:** Junfeng Qi and Wansen Li, Affiliated Central Hospital of Huanghuai University, No. 747, West Section of Zhonghua Road, Zhumadian 463000, Henan, China. Email: 20222297@huanghuai.edu.cn (Qi J). zcr3817202@163.com (Li W).

Introduction

Edible mushrooms have emerged as a globally significant alternative food source. Their consumption is projected to increase from 12.74 million tons in 2018 to 20.84 million tons by 2026, representing a compound annual growth rate of 6.41% [1]. Among these, shiitake mushrooms hold a prominent position in the market due to their distinctive sensory attributes, nutritional

value, and medicinal properties. As a nutrient-rich food, shiitake mushrooms are abundant in both micronutrients and macronutrients, making them key ingredients in dietary supplements. They also contain bioactive compounds including β-glucans and ergosterol along with trace nutrients including niacin, phosphorus, selenium, and copper [2]. These components offer potential health benefits such as cognitive protection and support for weight management as well as anti-cancer and anti-diabetes effects [3]. Fresh shiitake mushrooms are inherently susceptible to postharvest water loss and fungal decay, which significantly compromises their quality and shelf life. To address this issue, researchers have employed advanced preservation technologies including ozone treatment, ultraviolet irradiation, and modified-atmosphere packaging to extend shelf life of shiitake mushrooms while maintaining their nutritional value and food safety [4]. These efforts not only contribute to the sustainable development of the edible mushroom industry but also improve access to safe, nutritious food for consumers.

Edible nanogel, an innovative approach for preserving fresh fruits and vegetables, has been shown to significantly improve their texture and quality by effectively reducing transpiration and respiration rates [5]. According to international standards, edible nanogel is classified as an integral part of the food product, thereby ensuring their safe application [2]. In mushroom preservation research, a diverse array of natural bioactive compounds including essential oils, proteins, peptides, polysaccharides, and polyphenols has been incorporated into protective coatings. Notably, specific calcium alginate-based coatings exhibit exceptional mechanical and barrier properties, antibacterial, antioxidant, and antimicrobial activities [6]. These coatings contain active ingredients that interact with the sources of food spoilage, ultimately enhancing the overall quality and extending the shelf life of mushrooms. Marine polysaccharide κ-carrageenan, a member of the linearly sulfated galactan family derived from red seaweed [7], possesses unique properties that are highly beneficial for food packaging applications. As a water-soluble polysaccharide, κ-carrageenan can form gels and films under favorable conditions [8], making it an ideal candidate for edible nanogel formulations. Its inherent antioxidant properties further suggest potential applications in extending the shelf life of packaged foods. Notably, when combined with proteins particularly gelatin, κ-carrageenan significantly enhances the mechanical, thermal, and barrier properties of biopolymer films. These characteristics demonstrate κ-carrageenan's substantial significant potential as a natural edible nanogel material for improving postharvest quality in vegetables and fruits. This sustainable material shows promise for maintaining freshness and extending the freshness and shelf life of various agricultural products. Although carrageenan possesses numerous biological activities, its inherent hydrophilicity and subpar mechanical properties hinder its application in food preservation [9], thereby limiting its biological effectiveness when used alone for this purpose. To address these limitations, composite coatings based on carrageenan are currently being enhanced by incorporating natural ingredients including polysaccharides, polyphenols, and essential oils. Among these additives, polysaccharides demonstrate particularly good compatibility with carrageenan, enabling the formation of homogeneous composite structures, which makes polysaccharides especially suitable for creating blended coatings with carrageenan. The resulting composite materials show significantly improved mechanical properties along with enhanced antioxidant and antibacterial activities compared to pure carrageenan coatings.

The stipe waste generated during mushroom processing is often overlooked in traditional processing methods and contains about 20% polysaccharides [10]. Discarding this waste without proper treatment not only represents a loss of valuable agricultural resources but also poses potential ecological risks. Notably, studies have confirmed that mushroom stipe is rich in

bioactive compounds with antioxidant and antibacterial properties [11], suggesting broad application potential in food science. Specifically, it can be used as a natural additive in food production to enhance the preservative function of edible nanogel [12]. Therefore, this study aimed to comprehensively investigate the specific effects of a carrageenan-based coating fortified with *Lentinus edodes* stipe as an antioxidant on the postharvest quality of *Lentinus edodes* fruit bodies. The findings would provide scientific justification for agricultural waste utilization and innovation in food preservation technology.

Materials and methods

Mushroom stipe solution preparation

The mushroom stipe solution was prepared according to a modified method of Wang *et al*. [13]. Briefly, the mushroom stipe was mixed with boiling water in a ratio of 1:20 and homogenized into a paste using a high-speed blender. Subsequently, the paste was subjected to ultrasonic cell disruption for 6 minutes. The ultrasonic cell disruptor used the cavitation effect in the liquid to destroy the cell structure, thereby releasing the intracellular components. The optimized preparation method effectively obtained a mushroom stipe solution suitable for experimental applications.

Edible nanogel nanocoating solution preparation

Nanocoating solutions were prepared following established protocols [14]. Specifically, the adhesive solution (10% w/v) was formulated by heating at 40℃ for 60 min, dissolving carrageenan powder in distilled water, and then homogenizing the mixture with ultrasound for 6 hours. The solution was then purified through vacuum filtration. To enhance antioxidant and antibacterial properties, mushroom stipe was incorporated into the coating formulation with subsequent ultrasonic homogenization for an additional 30 s. The study comprised four distinct treatment groups designated as group C (control) being treated with distilled water, group K being formulated with a matrix of monocomponent containing 10% carrageenan aqueous solution (w/v), group M combined 10% carrageenan with 1.5% (v/v) mushroom stipe extract, group N featured 10% carrageenan supplemented with 3.0% (v/v) mushroom stipe extract.

Mushroom fruiting body treatment

The mushroom variety used in this study was the common 808 variety supplied by Henan Tianzhong Yilong Food Co., LTD (Biyang, Henan, China). The fruiting bodies were coated following a modified method derived from Tahir *et al*. [14]. Mushrooms were initially rinsed with distilled water at 60°C and then air-dried at room temperature for 1 hour. After air drying, the mushrooms were randomly divided into three treatment groups with 50 mushrooms in each group and being immersed in the respective coating solution for 4 minutes before allowing excess coating material to drip off naturally. The coated mushrooms were then placed on a polyethylene sheet and air-dried at 25 ± 1°C. Uncoated mushrooms rinsed with distilled water served as the control. Mushroom samples were packaged in 500 cm^3 polypropylene trays (Shantou Sanma Plastic Products Co., LTD., Shantou, Guangdong, China). Prior to storage, five 8-mm-diameter apertures were precision-aligned along the packaging's central axis with uniform equidistant spacing. The mushrooms were stored under refrigerated conditions at a temperature of 4 ± 0.5℃ and 75% humidity. At various intervals of 0, 4, 8, and 12 days during storage, a quality assessment was performed on 50 fruiting bodies of each batch. This process aimed to analyze and monitor the quality changes in the mushrooms over the course of the storage period.

Physical and chemical analysis

The physicochemical analysis was conducted using established protocols [15]. Bante220 pH/ORP Meter (Shanghai Shuang Xu Electronics Co., LTD, Shanghai, China) was employed to determine the pH value of the mushroom samples. A LLOYD TA1Plus texture analyzer

(Cloud Spectrum Instrument (Shanghai) Co., LTD, Shanghai, China) was used to assess the hardness of the fruiting bodies. The probe descended at 1 mm/s until it contacted the sample, and the recording began when the load reached the trigger point of 5 g. During texture analysis, the probe descended continuously with the load value increased until the set target of 2 mm was displaced. The probe was then retracted at the predetermined speed until it returned to its initial position, completing the test cycle. All data were automatically recorded. Total soluble solids (TSS) were measured using a RS232 refractometer (Nanjing Kejie Analytical Instruments Co., LTD., Nanjing, Jiangsu, China) at a temperature of 20°C. Weight loss was calculated as a percentage of the initial fresh weight determined using an analytical balance as follows.

$$\text{Weight loss rate} = \frac{(\text{initial weight} - \text{final weight})}{\text{initial weight}} \times 100\%$$

The parameters of pH, hardness, TSS, and weight loss were evaluated on days 0, 4, 8, and 12 of storage.

Decay ratio

The decay rate of the mushrooms was evaluated on days 0, 4, 8, and 12 of storage. The decay percentage was calculated based on visible damage and softening in injured areas of the shiitake mushrooms. Both coated and uncoated mushrooms were assessed as below.

$$\text{Decay percentage (\%)} = \frac{\text{Number of Rotten Mushrooms}}{\text{Total Number of Mushrooms}} \times 100\%$$

Microbial analysis

Microbial analysis was performed using a modified method described by Siroli *et al*. [16]. Briefly, shiitake fruiting bodies were homogenized using a high-speed blender. 10 grams of the homogenate was transferred to a sterile test tube and mixed with 90 mL of 0.9% sterile saline solution at room temperature. Serial dilutions of the shiitake mushroom homogenate were prepared, and aliquots were plated onto sterile potato dextrose agar (PGA) medium (Merck Sigma-Aldrich, St. Louis, MO, USA). The plates were incubated at 37°C for 48 hours, allowing for the growth and enumeration of colony-forming units (CFU) of thermophilic aerobic bacteria. Microbiological analyses were conducted in triplicate on storage days 4 and 12 to ensure reliability.

Total phenol determination

To extract the phenolic compounds from the shiitake mushrooms, a modified version of the method described by Tahir *et al*. was employed [15]. Briefly, shiitake fruiting bodies were homogenized using a high-speed blender with 10 mL of acidified methanol (0.1% HCl). The homogenate was wrapped in aluminum foil and stored at 4°C for 1 hour. After refrigeration, the mixture was centrifuged at 4°C and 12,000 g for 10 minutes. A 200 µL aliquot of the supernatant was transferred to a vial containing 2 mL of Folin-Ciocalteu reagent and thoroughly mixed using a vortex mixer. After 5 minutes, 2 mL of 10% Na_2CO_3 solution was added to the mixture and then incubated at 25°C for 1 hour. The absorbance of the solution was measured at 640 nm using a spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) with Gallic acid as a standard to generate calibration curves. The total phenolic content (TPC) was expressed as gallic acid equivalents (GAE) per 100 g of fresh weight (FW).

Determination of 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH) clearance

The DPPH free radical scavenging activity of the mushroom extracts was determined using a modified version of our previously published method [15]. Briefly, 0.2 mL of mushroom extract was mixed with 3 mL of methanolic DPPH solution (20 mg/L in methanol) and incubated in darkness for 30 minutes. The absorbance was measured at 517 nm using a spectrophotometer with 0.2 mL of shiitake mushroom extract mixed with 3 mL of methanol as a blank control and 3 mL of the DPPH solution mixed with 0.2 mL of

methanol as a negative control. The DPPH free radical scavenging rate was calculated as follows.

Scavenging Rate (%) = $[(A_c - A_f) / A_c] \times 100$

where A_c was the absorbance value of the negative control. A_f was the absorbance value of the test sample. Each experiment was conducted in triplicate to ensure accuracy and reliability of the results.

Total flavonoid content

The total flavonoid content (TFC) was measured using the methods described by Singh *et al*. [17]. 1 mL of the sample or standard was precisely diluted with 4 mL of distilled water, and 0.3 mL of 5% sodium nitrate solution was subsequently added. After incubation for 6 minutes, 0.3 mL of 10% aluminum chloride solution was added. The mixture was then incubated at room temperature for 5 minutes before 2 mL of 1M sodium hydroxide was added to the mixture. The mixture was thoroughly mixed, and the pink absorbance was measured at 510 nm using the spectrophotometer. The calibration curve was constructed using quercetin in the concentration range of 10 – 1,000 µg/mL, achieving a coefficient of determination (R^2) of 0.99. Total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dried extract (mg QE/g dry weight). All experiments were performed in triplicate to ensure reproducibility.

Superoxide dismutase determination

Superoxide dismutase (SOD) activity (u/g) of mushroom was determined using the superoxide dismutase kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) following provider's instructions.

Statistical analysis

All experiments were conducted with three technical replicates and three harvests with three biological replicates. The results were statistically analyzed using Analysis of variance (ANOVA). Minimum significant difference (LSD) was calculated to compare the significance of the 5% level ($\alpha = 0.05$). All the data were generated using OriginPro software (OriginLab Corporation, Northampton, MA, USA).

Results and discussion

Effect of edible nanogel coating on postharvest quality of mushroom

(1) Physicochemical properties of mushroom stipe nanogels

The physicochemical properties of edible nanogel materials fortified with mushroom stipe were shown in Figure 1. A significant difference in pH was observed among the carrageenan-based edible nanogel containing varying concentrations of mushroom stipe ($P < 0.05$). Specifically, the nanogel coating with 3.0% mushroom stipe (N) had the highest pH of 4.98 and hardness of 1,004.76 on the 12th day of storage followed by the coating with a lower concentration (M) at the pH of 4.79 and hardness of 1,004.04, respectively. Conversely, the water-treated control group exhibited the lowest pH of 4.46 and hardness (1,000.21). Notably, as the concentration of mushroom stipe increased, the pH of the edible nanogel coating material increased within the range of 4.46 to 4.98, and the hardness increased within the range of 1,000.21 to 1,004.76. On the 12th day of postharvest storage, the total soluble solids (TSS) value of the edible nanogel group increased proportionately with the increasing concentration of mushroom stipe. The results showed that the incorporation of mushroom stipe into the gel formulation significantly influenced the TSS value ($P < 0.05$) (Figure 2A). Specifically, the 3.0% stipe gel (N) showed a higher TSS value of 1.55, while a lower TSS value was noted at a concentration of 1.5%. Furthermore, the mushroom stipe significantly affected the weight loss rate of the gel coating ($P < 0.05$) (Figure 2B). As the concentration of mushroom stipe increased, the weight loss rate of the edible nanogel coating materials decreased, ranging from 9.93% to 19.37%. The results demonstrated that the pH values of the gel coating materials were significantly correlated, whereas the TSS increased with the

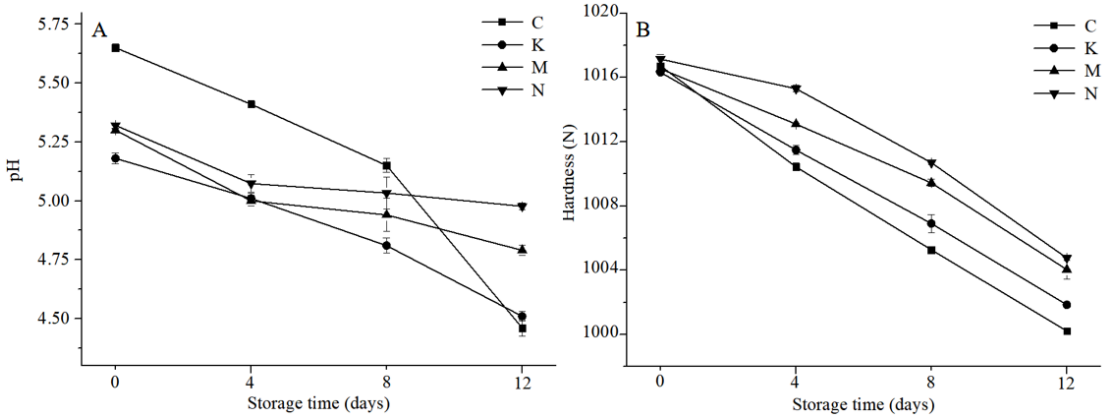

Figure 1. Effect of edible nanogel coating on pH (**A**) and hardness (**B**) of mushroom. C: control. K: 10% carrageenan gel. M: 10% carrageenan gel + 1.5% (v/v) mushroom stipe contents. N: 10% carrageenan gel + 3.0% (v/v) mushroom stipe contents.

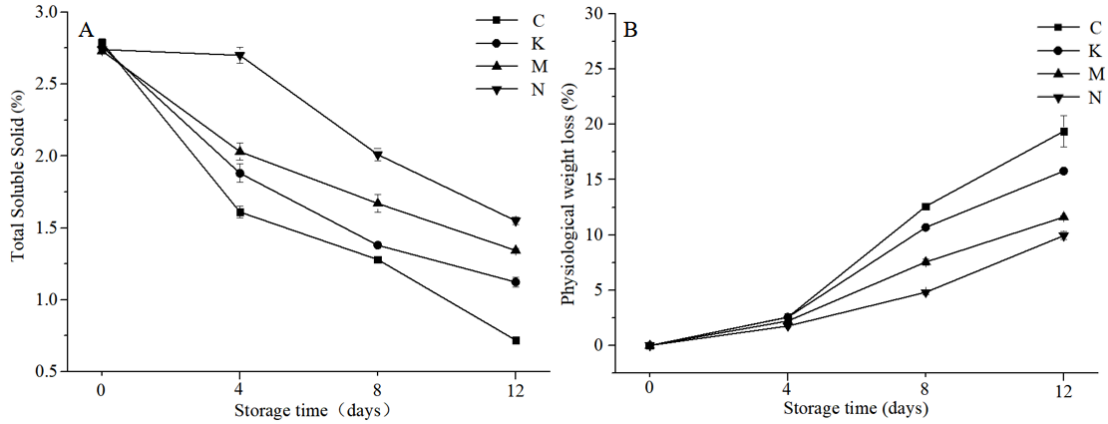

Figure 2. Effect of edible nanogel coating on total soluble solid (**A**) and weight loss rate (**B**) of mushroom. C: control. K: 10% carrageenan gel. M: 10% carrageenan gel + 1.5% (v/v) mushroom stipe contents. N: 10% carrageenan gel + 3.0% (v/v) mushroom stipe contents.

increasing concentration of mushroom stipe, which likely reflected increased material hydrophobicity. These results highlighted water activity's critical role as a crucial factor in assessing the storage quality of edible fungi. Freshly harvested shiitake mushrooms possess high water content compared to other crops [18], which can lead to rapid microbial proliferation [19]. Additionally, the unprotected epidermal structure and high transpiration rate of edible fungi render them vulnerable to water loss, wilting, decay, and browning during storage [20]. The elevated hardness and TSS values of the gel coating containing mushroom mycorrhiza were associated with water loss in the samples [21]. These findings aligned with previous research by Yazicioglu *et al*. who reported that the application of guar gum coating reduced the weight loss of postharvest edible fungi [22].

(2) The mushroom stems help to maintain quality

Some previous studies suggested that various coating materials including chitosan, gum, agar, egg white protein, lecithin, monoester, gum Arabic, nanocellulose, sodium alginate/modified starch, nano-silver sodium alginate, and pectin could effectively delay physiological weight loss in mushrooms during storage at different temperatures [23-28]. These coatings formed a semi-permeable layer between the mushroom surface and the external environment, thereby

reducing water loss and extending mushroom shelf life. Studies have demonstrated that such coatings represent an effective strategy for improving mushroom storage quality. During the entire 12-day refrigerated storage period, the postharvest decay rate of mushroom fruiting bodies exhibited a decreasing trend, irrespective of whether antioxidant-enriched mushroom stipe was added. At the end of storage, the decay rate of the control samples (without mushroom stipe) reached a minimum value of 53.33%. In contrast, nanogel-coated fruits showed a 13.33% lower decay rate compared to nanogel-coated samples without mushroom stipe (Figure 3). Increasing concentrations of mushroom stipe in the edible nanogel were correlated with decreasing mushroom decay rates, which might be attributed to the permeability created between the mushroom surface and the edible nanogel to aid in reducing the respiration rate and water loss. The absence of a cuticle on shiitake mushrooms surfaces leads to rapid dehydration and mass transfer, explaining increased fruit decay rates for uncoated samples over time. The edible nanogel serves as a semi-permeable film that mitigates water loss and regulates gas exchange. The results aligned with previous studies by Athipornchai *et al*. who demonstrated that carrageenan gel acted as a barrier to water transfer and inhibited fruit rot rates in coated oranges [8]. Similarly, Riahi *et al*. reported a reduced decay rate in shrimp coated with carrageenan [29]. These results confirmed that edible nanogel based on carrageenan gel could reduce the respiration rate of mushrooms by acting as a semi-permeable barrier against water loss, ultimately leading to a lower decay rate. The number of microorganisms in samples coated with the mushroom stipe edible nanogel material was significantly lower than that in uncoated samples during the storage period ($P < 0.05$). With increasing mushroom stipe concentration in the coating, the microbial counts showed a decreasing trend (Table 1). Specifically, the sample coated with 3.0% mushroom stipe exhibited a microbial count of 3.80 logCFU/g at the end of storage with a substantial reduction compared to other coated samples. The antimicrobial efficacy against mushrooms increased with higher mushroom stipe concentrations in the gel coating. This reduction in microbial load might be attributed to the antioxidant properties of the mushroom stipe gel, which acted on the mushroom surface to inhibit microorganism reproduction. The findings of this microbiological analysis aligned closely with those reported by Yazicioglu *et al*. who developed an edible nanogel composed of leek powder/sunflower oil in a guar gum base and applied it to button mushrooms [22]. Their study indicated that the leek powder/sunflower oil combination could effectively suppress microbial proliferation on fruits by inhibiting water loss and gas exchange. Additionally, Yang *et al*. developed an edible coating containing natural preservatives such as baobab seed oil, which was shown to reduce respiratory rates and inhibit microbial growth [30].

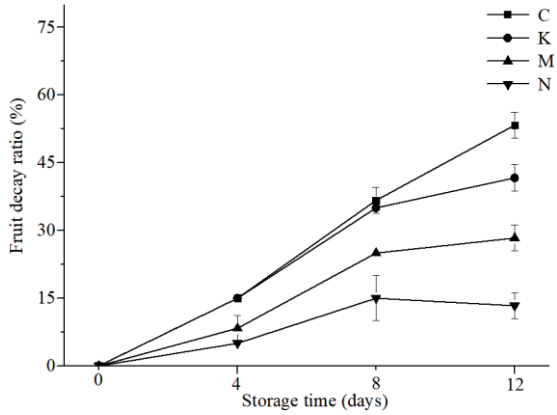

Figure 3. Effect of edible nanogel coating on fruit decay rate of mushroom. C: control. K: 10% carrageenan gel. M: 10% carrageenan gel + 1.5% (v/v) mushroom stipe contents. N: 10% carrageenan gel + 3.0% (v/v) mushroom stipe contents.

Mushroom postharvest total phenol, DPPH clearance, flavonoids and superoxide dismutase

The total phenol content of all samples was affected by the concentration of mushroom stipe gel ($P < 0.05$). At the end of storage, samples M and N exhibited higher phenolic contents than samples K and control C (Figure 4). The low phenol content in some samples might result from polyphenol utilization in brown pigment

Table 1. Effects of edible nanogel coatings on microbial populations (mean ± standard deviation).

Treatments	Microbial population (log CFU/g)			
	4 days storage		12 days storage	
	Mean ± standard deviation	*P* value	Mean ± standard deviation	*P* value
C	4.17 ± 0.06	-	4.88 ± 0.08	-
K	3.81 ± 0.06	0.000*	4.34 ± 0.08	0.000*
M	3.59 ± 0.06	0.006*	4.07 ± 0.06	0.009*
N	3.28 ± 0.05	0.000*	3.80 ± 0.07	0.008*

Notes: * indicated the significant differences between the samples.

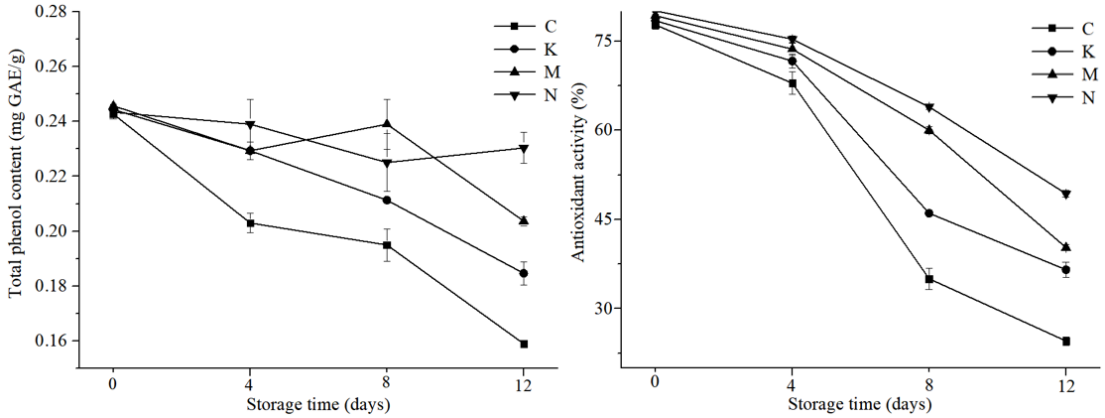

Figure 4. Effects of edible nanogel coating on total phenol (**A**) and DPPH clearance (**B**) of mushroom. C: control. K: 10% carrageenan gel. M: 10% carrageenan gel + 1.5% (v/v) mushroom stipe contents. N: 10% carrageenan gel + 3.0% (v/v) mushroom stipe contents.

synthesis. The incorporation of mushroom stipe in carrageenan based food coatings at varying concentrations significantly enhanced DPPH radical scavenging activity in mushrooms throughout storage. Samples in N group treated with 10% carrageenan solution + 3.0% mushroom stipe solution exhibited higher scavenging activity than those in groups M, C, and K (Figure 4B), indicating that the increase of mushroom stipe concentration in the edible nanogel of mushroom stipe gel significantly increased the DPPH clearance rate. Phenolic compounds are reported to play a crucial role in antioxidant defense by scavenging free radicals and terminating free radical chain reactions. The results of phenol content in this study were consistent with previous findings by Kumar *et al*. who reported that the edible coating of aloe vera gel kept the phenolic content of the treated bisporal mushrooms high compared to controls [26]. This result has been widely applied to the phenolic parts of fruits and vegetables [5]. Shan *et al*. also found that wild edible fungi (*Boletus bicolor*) treated with a sodium alginate composite coating of green tea extract crosslinked gelatin retained higher phenolic content during storage at 4°C for up to 7 days [31]. In addition, Qu *et al*. investigated the effects of different concentrations of peppermint oil on the postharvest quality of white mushroom (*Agaricus bisporus*) and found that increasing the concentration of essential oil significantly increased the antioxidant capacity of mushrooms during storage [32]. Consistent results were reported by Nasiri *et al*. who found that chitosan and glucose complexes, tragacanthus containing *Zataria multiflora* essential oil, and a chitosan coating covered with white cauliflower had potential effects on the phenol content of bispora agaricus [33]. These findings helped to enhance the understanding of the helps to enhance the antioxidant activity of mushrooms

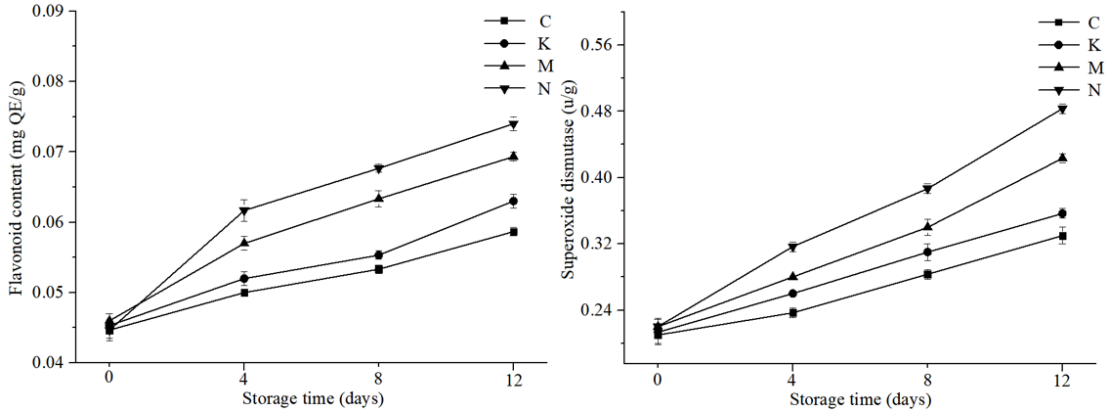

Figure 5. Effects of edible nanogel coating on flavonoids (**A**) and superoxide dismutase (**B**) of mushroom. C: control. K: 10% carrageenan gel. M: 10% carrageenan gel + 1.5% (v/v) mushroom stipe contents. N: 10% carrageenan gel + 3.0% (v/v) mushroom stipe contents.

and minimize their oxidation risk. The findings of DPPH clearance were consistent with previous research by Guo *et al*. who retained the higher antioxidant activity of shiitake mushrooms using an edible coating of lentinan [12]. This might be due to the increased activity of phenylalanine ammoniase and the continued accumulation of phenolic components in mushrooms. Liu *et al*. also maintained high antioxidant activity of the fruit bodies of *Lentinus edodes* by adding *Oudemansiella radicata* polysaccharide and storing it at 4°C for up to 18 days [34]. Therefore, adding polysaccharide containing substances to edible coating could also improve the antioxidant capacity of mushroom coating materials by delaying oxidative degradation [35]. Flavonoids play a vital role in the antioxidant systems of fruits and vegetables through their scavenging activity. During the 12-day storage period, the flavonoid content and associated antioxidant activity were significantly affected by mushroom stipe gel concentration ($P < 0.05$) (Figure 5A). Mushrooms coated with the mushroom stipe gel retained higher flavonoid content than control samples. Among all treatments, the samples of N group had the highest flavonoid content at the end of storage. The same results were observed in mushrooms coated with alginate-based coatings [36]. It was also found that the gel with 3.0% edible nanogel containing the mushroom stipe was most likely to retain higher SOD activity in the mushroom samples throughout the storage period (Figure 5B). SOD activity in *L. edodes* increased during storage, peaking at day 12, and N group showed the highest superoxide dismutase activity of 0.48 at the end of the storage period. This result was very much in line with previous findings by Sun *et al*. who treated oyster mushrooms with chitosan/hypersphenated polysine and observed that coated samples retained high superoxide dismutase activity [37]. This phenomenon might be attributed to the mushroom stipe gel inducing a defensive response against external stress during storage, which formed a barrier against water and oxygen exchange while enhancing overall antioxidant capacity.

During the postharvest period, the absence of protective epidermal tissue and high moisture content in edible fungi facilitates microbial colonization, leading to enzymatic activity changes. Common contaminating bacteria include *Pseudomonas tola*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Listeria monocytogenes*. In addition, mold is another microbe that can cause edible fungus infection [38], resulting in adverse changes in plant texture, taste, or odor, and may lead to reduced hardness, total soluble solids [39], total phenols, flavonoids, and activated superoxide dismutase [40]. Lentinan (LEP) is a natural polysaccharide from the mushroom stipe and has a wide range of effects such as antioxidant and hypoglycemic

effects. By providing an effective external environmental barrier, it can improve the preservation of mushrooms, delay the decay and improve the postharvest quality. Guo *et al*. investigated the effect of polysaccharide coating on the browning and softening of mushroom and showed that LEP increased the activity of multiple antioxidant enzymes and significantly reduced the accumulation of hydrogen peroxide compared to the control [38]. In addition, during storage, LEP treatment maintained the high antioxidant activity of mushrooms and inhibited the activity of brown-related enzymes like polyphenol oxidase and tyrosinase, reducing browning. Further, it maintains high levels of cellulase, chitinase, and β-1,3 glucanase to improve softening during storage [12].

Conclusion

In this study the shelf life of shiitake mushrooms was successfully extended by using an edible carrageenan-based nanogel coating either pure or supplemented with varying concentrations of shiitake mushroom stipe extract to maintain postharvest quality and sensory properties during storage at 4°C. The carrageenan-based edible nanogel coating, particularly when supplemented with mushroom stipe extract, demonstrated the potential to reduce respiration rates and inhibit water loss in treated mushrooms. Coated mushrooms showed significantly better maintenance of hardness, total soluble solids, total phenol content, antioxidant activity, and total flavonoid content compared to uncoated control samples. Among all coating treatments, carrageenan gel with 3% *Lentinus* mushroom stipe extract was most effective in retarding microbial growth and maintaining superior quality throughout storage. Therefore, a double-layer edible nanogel, i.e. carrageenan gel mixed with mushroom stipe, could be an alternative and less expensive method to extend the shelf life of mushrooms up to 12 days when compared to uncoated samples. Further research will focus on developing smart nano-packaging systems incorporating lentinan gel to enhance barrier properties to gas and moisture exchange, thereby further extending storage time while maintaining product quality.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No. 22406050), the Science and Technology Research Project of Henan Province, China (Grant No. 242102110140, 242102231059, 252102321121, 242103810011), the Science and Technology Innovation Youth Special Project of Zhumadian, Henan, China (Grant No. QNZX202319), the Major Science and Technology Projects in Zhumadian City (Grant No. ZMDSZDZX2024010), Breeding of New Varieties of Mushroom and Research and Development of Deep-processing Products Related to Mushroom (Grant No. 2024411701000014), Research on Improving Bottom Sediments and Ensuring Water Quality Using Biochemical Method In Aquaculture (Grant No. 2024411701000085), Fungal Mushroom Strain Development, Bioactive Compound Extraction, And Functional Mushroom Product Innovation (Grant No. 2023411701000060).

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